

**G $\alpha$ 12 and G $\alpha$ 13 Subunits Define a Fourth Class of G Protein  $\alpha$  Subunits**

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Notes:

## Gα12 and Gα13 subunits define a fourth class of G protein α subunits

(GTP-binding protein/signal transduction)

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**ABSTRACT** Heterotrimeric guanine nucleotide-binding regulatory proteins (G proteins) are central to the signaling processes of multicellular organisms. We have explored the diversity of the G protein subunits in mammals and found evidence for a large family of genes that encode the α subunits. Amino acid sequence comparisons show that the different α subunits fall into at least three classes. These classes have been conserved in animals separated by considerable evolutionary distances; they are present in mammals, *Drosophila*, and nematodes. We have now obtained cDNA clones encoding two murine α subunits, Gα12 and Gα13, that define a fourth class. The translation products are predicted to have molecular masses of 44 kDa and to be insensitive to ADP-ribosylation by pertussis toxin. They share 67% amino acid sequence identity with each other and <45% identity with other α subunits. Their transcripts can be detected in every tissue examined, although the relative levels of the Gα13 message appear somewhat variable.

Guanine nucleotide-binding regulatory proteins (G proteins) are heterotrimers composed of α, β, and γ subunits (for reviews see refs. 1–3). The α subunits belong to a much larger group of GTPases, including elongation factor Tu and Ras, which share similar structural elements (4, 5). In all of these GTPases, a cycle of guanine nucleotide exchange and hydrolysis enables the protein to exist in two distinct states. This cycle allows G proteins to transiently relay signals from cell-surface receptors to intracellular effectors. The receptors comprise a diverse family of proteins characterized by their transmembrane structure; they have seven membrane-spanning domains with highly conserved amino acid sequences. Upon interaction with the appropriate agonist, the receptor serves to accelerate the exchange of GDP for GTP on the G protein α subunit. This exchange is believed to be accompanied by dissociation of the α and β-γ subunits, allowing α (and in some cases β-γ) to interact with effectors. The intrinsic GTPase activity terminates the signal, returning the α subunit to its basal GDP-bound state.

More than 100 different receptors in mammals are thought to couple through G proteins to a variety of effectors (6). The diverse α subunits, which mediate receptor function, can be classified on the basis of their amino acid sequence similarity. Stimulatory (G<sub>s</sub>) and inhibitory (G<sub>i</sub>) G protein subtypes have been implicated in the regulation of adenylate cyclase and the gating of certain ion channels (7). In the highly specialized visual system, biochemical experiments have elucidated the role of transducin (G<sub>ti</sub>) in regulating phosphodiesterase and subsequently in controlling the levels of cGMP during the photoreceptor cascade (8). These systems are the best characterized examples of G protein-mediated effector activa-

tion. However, many other effector systems have been shown to be regulated by G proteins.

Pertussis toxin has proven to be an important tool in the dissection of G protein-mediated pathways. Certain α subunits can be ADP-ribosylated by this toxin, thereby uncoupling the G protein from receptors. There are, however, some signaling processes that are refractory to toxin inhibition but seem to be mediated by G proteins. In particular, phospholipase C (PLC) is involved in both pertussis toxin-sensitive and -insensitive pathways (9). Recently, using molecular biological techniques we found a class of α subunits termed G<sub>q</sub> that were predicted to be insensitive to pertussis toxin (10). Two groups independently purified the corresponding α subunits and have now demonstrated that these proteins can activate one of the PLC isotypes, PLC-β (11–13).

We are interested in understanding how G protein-mediated signal transduction has adapted to the complex signaling processes that define multicellular organisms. To this end, we have examined G protein diversity in the mouse. Using a method based on the PCR, we found evidence for extensive diversity among the G protein α subunits (14). A small screen uncovered five sequences termed Gα10–Gα14. Analysis of amino acid sequence conservation suggested that the known α subunits could be grouped into three distinct classes, G<sub>s</sub>, G<sub>i</sub>, and G<sub>q</sub> (10). Gα11 and Gα14 belong to the G<sub>q</sub> class. In this paper, we present the cDNA sequences of Gα12 and Gα13, which define the fourth class of α subunits.\*

### MATERIALS AND METHODS

**PCR.** cDNA was made from 5 μg of total RNA with random hexanucleotide primers using Moloney murine leukemia virus reverse transcriptase in a 50-μl reaction volume. Conditions were those supplied by the manufacturer (Bethesda Research Laboratories). The reactions were heated to 100°C for 5 min. The cDNA was then diluted to 400 μl with water containing DNase-free RNase A at 10 μg/ml and was incubated at 37°C for 30 min. Two microliters of the cDNA mixture was used in a 20-μl PCR reaction. The oligonucleotides used for PCR amplification of the cDNA were as follows: *oMP19*, CGGATCCAARTGGATHCAYTGTT; *oMP20*, GGAATTCRTCCTTCTTTRTTNAGRAA; *oMP21*, GGAATTCRTCCTTCTTTRTTTAAARAA; and *CT56*, CGGATCCARRTGGHTNSARTGYTT, in which R = A or G, Y = C or T, S = C or G, H = A, C, or T, and N = A, C, G, or T. PCR was performed for 35 cycles on a Perkin-Elmer/Cetus thermal cycler. During each cycle, samples were denatured for 0.5 min at 94°C, annealed for 0.5 min at 40°C,

Abbreviations: PLC, phospholipase C; G protein, guanine nucleotide-binding regulatory protein; G<sub>s</sub> and G<sub>i</sub>, stimulatory and inhibitory G protein, respectively; G<sub>q</sub>, class of G protein α subunits predicted to be insensitive to pertussis toxin.

\*The sequences reported in this paper have been deposited in the GenBank data base [accession nos. M63659 (Gα12) and M63660 (Gα13)].

and extended for 0.5 min at 72°C. Each oligonucleotide was used in the PCR at 10 ng/μl. The buffer and *Taq* polymerase were supplied by Cetus.

**PCR RNA Analysis.** PCR was performed on cDNA prepared from total RNA, as described above. To control for possible contamination of the RNAs by chromosomal DNA, we treated each preparation of RNA as described above, except reverse transcriptase was not added to the cDNA synthesis mixture. These controls were then subjected to PCR alongside the other samples. The PCR products were electrophoresed through a 2% agarose gel, blotted to Hybond-N filter (Amersham), and hybridized according to the manufacturer's instructions. These blots were probed with

radiolabeled oligonucleotides specific to *Gα11* (CTCGCT-TAGTGCCACC), *Gα12* (CTCGCTCGAGGACACCAT-GAAC), and *Gα13* (TTCATTGAAGAGACAAGGAAA). The oligonucleotides were end-labeled with [ $\gamma$ -<sup>32</sup>P]ATP, as described (15). Blots were washed three times at room temperature for 5 min in 0.90 M NaCl/0.90 M sodium citrate (6× SSC)/0.1% SDS followed by a 1-min wash in the same solution at the calculated melting temperature of each oligonucleotide.

**Isolation of cDNAs.** To obtain cDNAs encoding *Gα12* and *Gα13*, a random-hexanucleotide-primed mouse brain cDNA library in λZAPII (Stratagene) was screened by using standard techniques. Six hundred thousand clones were plated at

## Gα12

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TCGGGGCGGGCGGG -121
CGGACGCGAGCTGAGGAGCGGGCGGGCGGGCGGCGCTGGCCGCGAGGCGCGCGCGG -61
CTGAGGGCGCTCATGGCGCGGGCGCCAGCGGGCGGGCGGGCGGCTGAGGGCGGCG -1
ATGTCGGGGTGGTGGCGGACCTTAGCCGCTGCTGCTGCGCGCGAGGCGGAGCGCGC 60
M S G V V R T L S R C L L P A E A G A R 20
GAGCGGAGGCGGGCGGGCGGCGGAGCGCGGAGGCGGAGCGGCGAGCGCGGCGGAC 120
E R R A G A R A D A E R E A R R R S R D 40
ATCGACGCGCTGCTGGCGCGGAGCGGGCGGGTGGCGGCGGCTGGTCAAGATCTCTGCTG 180
I D A L L A R E R R A V R R L V K I L L 60
CTGGGCGCGGCGGAGCGGCAAGTCCACTTCTCAAGCAGATGCGCATCTCCACGGC 240
L G A G E S G K S T F L Q M R I C H G 80
CGGAGTTCGACGAGAGCGCTGCTGGAGTTCGCGGACACCATCTTCGACAACTCCTT 300
R E F D Q K A L L E F R D T I F D N I L 100
AAGGGTTCGAGGGTCTTGTGGACGCTCGAGACAGCTCGGCTCCCTGGCAGCACTCT 360
K G S R V L V D P A K L G I P W Q H S 120
GAGAAGGAGAGCAGCGGATGTTTCTGATGGCTTCGAGAACAGCGAGGCTGCCTGTG 420
E N E K H G M F L M A F E N K A G L P V 140
GAGCCTGCCACCTTCCAGCTTACGTGCGGCTGAGTCCCTCTGGAGAGACTCGGGG 480
E P A T L Q P A I R L W R D S G 160
ATCAGGGAAGCTTCAGCGCGAGAGCGAGTTCAGCTGGGTGAATCAGTGAAGTACTTC 540
I R E A F S R R S E F L G E S V K Y F 180
CTGGATAACTTGGACCGGATGGCCAGCTGAAGTACTTCCCGAGTAAAGCAAGCACTCTG 600
L D N L D R I G Q L N Y F P S K Q D I L 200
CTGGCTAGAAAGGCCACCAAGGAATCGTGAACATGACTTCGTTATAAAGAAATCCCA 660
L A R K A T K G I V E H D F V I K K I P 220
TTAAGATGGTGGATGTGGGCGGCGAGGTCACAGCGCGAGAGTGGTTCAGTGCCTTC 720
F K M V D V G Q R K W F Q C F 240
GACGGCATCATCTATCTCTGTTTCTGCTCGAGCGAGTATGACAGGTCCTCATG 780
D G I T S I L F M V S S S E Y D Q V L M 260
GAGGACAGGCGCAACCGGCTGGTGGATGCAATCTCTGAGACCATCGTCAAC 840
E D R R T N R L F E S M N I F E T I V N 280
AACAAGCTCTTCTTCAAGCTTCCATCATCTCTTCTTCAACAAGATGGACCTCTGGTG 900
N K L F T F N V S I I L F L N K M D L L V 300
GAGAAGTGAAGTCTGTGAGCACTTAAAGACCTTCCAGATTCAAGGCGGCGGCGAC 960
E K V K S V S I K K H F P D F K G D P H 320
CGGCTGGAGGACGCTCCAGCGCTACCTGGTGCAGTCTTCGACAGGAAGCGAGAACGCG 1020
R L E D V Q R Y L V Q C F D R K R R N R 340
AGCAAGCCCTGTTCACCACTTCCACCGCCGACACCGGAGAACCTCCGCTTCGTG 1080
S K P L F H H F T T A I D E N I R F V 360
TTTACGTGTGTAAGGACACGATCTCGAGGAGAACCTGAAAGACATCATGCTGCAGTGA 1140
F H A V K D T I L Q E N L K D I M L Q 379
GAGACAGCCCTCCCTCTGCTGCTCTCGGCTCTGGGCTGTGGCGCGCCACGTTGGT 1200
CTGGTGTGCTGCGCGCTGATGAGCTGAGTGGGCTCTGGAATCCACACCATCTAAT 1260
ACCTGGCTCAAGAGTGTGCTGACTACGACGACACGAGCGAGCTCGGGCAAGGAGCGCG 1320
GGAGTGTGAGTGTGATGCTGAATCGGGGACTGTGAGACTACTGAATCCACACCATCAC 1380
CTGCTGTGATCTGTAAACCTGGGGACACAGCTTTCGCTCTCTACAGAACTCTTTC 1440
TTGTTGACACAGTTAAACCAAGGTTACGCTGACGACACACGCGTCTCTCAATTAATG 1500
ACAGAAACCAACCGGCTGGCTTTCAGCGCGGCTCTCCCTCTGCGGTGCAAGTCTCT 1560
ACTGAGTCACACTAACTCGGAGCTTGGCTGACACTAGGCTGTAAAGAGCATGCGATG 1620
TGCTAAGCTACCGTGTCCAGGCGGCGCTCAGTGGCAGCTTCCAGAGTGAACACAG 1680
COTTAGCATGCTGCCAGCACCTCCGAGTCTCGGAAAGGCGAGGCGGGGTGAGTGTCT 1740
ACACT 1745

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## Gα13

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TCCCTCCC -121
CGGGGCGGCTCGGGCGCGGCTGCGCTCCCGGCTCCAGCGGCTTGGCGAGCGCGCC -61
GCTCCGGAGGAGGAGTGGAGGAGCGGAGGCGCGGCGGCGGCGGCGGCGGCGGCGGAG -1
ATGGCGGACTTCTGCGCTGCGCTGCGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 60
M A D F L P S R S V L S V C F P G C V L 20
ACGAAGCGAGGCGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAG 120
T N G E A E Q Q R K S I H G I D K C L S R A 40
GAGAAGACTTACGTGAAGCGGCTGGTGAAGTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 180
E K T Y V K R L V K I L L L G A G E S G 60
AAGTCCACTTCTTGAAGCAGATGCGGATCATCCAGCGGCGAGGACTTCCAGCGCGCGG 240
K S T F L K Q M R I H G Q D F D Q R A 80
CGCGAGGAGTTCGCGGCGGACCATCTACAGCAACGTGATCAAGAGTATGAGGCTGCTGTA 300
R E E F R P T I Y S N V I K G M R V L V 100
GATGCCGAGAGAGCTTCAATATCCCTGGGAGATAACAAAACAGCTTCCATCGAGAGC 360
D A R E K L H I P W G D K N A N L H G D 120
AAGTTGATGGCTTGTATACCGCGCGGCGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 420
K L M A F D T R A P M A A Q G M V E T R 140
GTGTTCTGCTGAGTATCTTCTGCTATCAGAGCCTTATGGGAGGACAGTGGTATCAGAA 480
V F L Q P A I R A L W E D S G I Q N 160
GCCTACGATCGGCGCGGGAATTCAGCTGGGTGAGTCTGTAAAGTATTCTTGGATAAC 540
A Y D R R R E F Q L G E S V K Y F L D N 180
TTGGATAAACTGGAGTACCGGATACATCCATCAAGATCAAGATATCTGCTTCCGAGA 600
L D K L G V P D Y I P S Q Q D I L L A R 200
AGGCCACCAAGGACCTCATGAGTACGACTTTGAAATTAAGAAATGTTCTTCAAAATG 660
R P T K G I H E Y D F E I K N V P F K M 220
GTTGATGTAAGTGGCGAGATCAGAACGGAACGCTGGTGTGAATTTGACAGTGTG 720
V D V G G Q R S E R K R W F E C F D S V 240
ACGTGATCTTCTTCTGCTCTTCAAGTGAATTTGACAGGCTGCTTATGGAGGACCGC 780
T S I L F L V S S S E F D Q V L M E D R 260
CAGACCAACTCGCTTACAGAACTCTGAACTTTTGAACAAATTTGCAACAACTCGGGT 840
Q T N R L T E S L N I F E T I V N N R V 280
TTCAGCAAGCTTCCATAATCTCTTCTTAAACAGAGAGACTTGTCTGAGGAGAAAGTG 900
F S N V S I I L F L N K T D L L E E K V 300
CAAGTTGTAGCTCAAGACTATTCTTGAATTTGAAGGAGGACCGGCTGCTTAAAG 960
Q V V S I K D Y F L E F E G D P H C L R 320
GACGTCCAAAGTTTCTGGTGAATGCTTCGGGGGAGAACCGGCGGAGCAGCAGAGG 1020
D V Q K F L V E C F R G K R R D Q Q Q R 340
CCGTGTACCACTTCAACCGCGGATCAACAGAGAGAACTCGGCTCGTGTTCGGG 1080
P L Y H H F T T A I N T E N I R L V F R 360
GACGTGAAGGACAGATCTTATGACAACTGAAGCAGCTCATGCTGAGTGATGACAA 1140
D V K D T I L H D N L K Q L M L Q 377
GACTCGCTGTTTAAATACCTTGTGTGATTTGATGTTTCTGTTTGTCTGTTTCTTCT 1200
CTTTAAATGGCAGTTTACACAGCAGTTAGAAGATCTCAGTTGTGTGTGTGTTAACTTCT 1260
TGGAGAACTTGTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 1320
TTGCCAATATCTGTAGAAATTTGGGCTTTGATTTGCTGCTGCTGCTGCTGCTGCTGCTGCT 1380
CAATTAATCTTCTGCAACCTTACACTAGTGTGTTTTCAGGCTGTAATCTCTAATC 1440
TGCTCTGCACTTAAAGAGAGAGAGTGCCTTACCGAGCAATGACATGCTTCTGCTGCTGCT 1500
CTGCTGCTGCAAGGCTGTTAGAGAAAGTTTGAAGAGAGTCTGAGGAGTGAAGATGAA 1560
TGGCCCCCTACACACAGCAGTGTGTTTTCCTCAATTTCTCTAATTTCTGCTGAGG 1620
CAGCAGTCTCAGGCGGAGGCTTGGCGGCGGCTACACACAGCAGTGTGTTTCTGCTGCT 1680
CAATCTCTTACTTCTGAAGTAACTCTTACTGCTTAAAGTTTACAAAGTGAATGCTCAG 1740
CCTGATGCTGATGCGGTCAAAATGGTATGCTGCTATGTTGCTGATGATGAGCAGTGA 1800
CAGCAGTCTTCTTTCGATACATGCTGCTCACTAACTTAAATGCTGCTGTTACAACTCT 1860
GGAGAGGCGAAGCACTGCTGAGGAGCATCACTACAGAGCGGCGGAGTGTGCTGCTTCT 1920
GGTCTGCTCTCTTGGACTTGTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 1980
TTTGCCCACTGCTCAACTCAGCAGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 2040
CCCCGAGAGTTTGGTGGGCGAGTTTCCCTGTGAAGTGTTCAAAAGGCACTCTTCTGCT 2100
ACCTGTCCTGTTAGTGTCTCTGTGACAGAGTTTGTGTTGTTGTTGTTGTTGTTGTTGTTG 2160
TGTGTGAGACAGCTTCTGAGGTACATCAATGTCTTAAAGTGCATCTAAGGATA 2220
AGCAATTTTTTTTTT 2294

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FIG. 1. Nucleotide sequence and predicted amino acid sequence of *Gα12* and *Gα13*. The complete sequences of two cDNA clones encoding *Gα12* and *Gα13* (rG12-5 and 3-G13, respectively) are shown. These clones were isolated from a random-primed mouse brain cDNA library. Sequence was obtained from two other clones (rG12-1 and rG12-2), which begin at bases 5 and -100, respectively, of the *Gα12* sequence. Two hundred bases from the 5' ends of both clones were sequenced and found identical to rG12-5.

a density of  $3 \times 10^5$  plaques per 150-mm Petri dish (16). Nylon filters (Hybond-N) were prepared and hybridized according to the manufacturer's instructions. Probes were made from the  $G\alpha 12$  and  $G\alpha 13$  PCR fragments isolated previously. These fragments were labeled by using the Multiprime DNA labeling system (Amersham).

**Sequence Analysis.** rG12-5 and 3-G13 cDNA clones (Fig. 1) were subcloned into plasmid pMOB (17). To obtain sequence priming sites spaced throughout the clones, the  $\gamma\delta$  transposon was randomly inserted into the cDNAs, and a subset of these insertions spaced every 300–400 bases along the length in each clone was isolated by previously described methods (17). Supercoiled plasmid DNA was sequenced by using the Sequenase kit marketed by United States Biochemical.

## RESULTS

**Sequence of  $G\alpha 12$  and  $G\alpha 13$ .**  $G\alpha 12$  and  $G\alpha 13$  were initially isolated as PCR products. Mouse brain cDNA was amplified with a set of degenerate oligonucleotide primers corresponding to amino acid domains conserved in all  $\alpha$  subunits (14). The resulting mixture of PCR products was characterized by sequencing individual clones.

To obtain full-length cDNAs encoding  $G\alpha 12$  and  $G\alpha 13$ ,  $\approx 6 \times 10^5$  clones from a random-primed mouse brain cDNA library were screened at high stringency by using the individual PCR products as probes. Twenty-five positive clones were obtained with the  $G\alpha 12$  probe, whereas four were found using the  $G\alpha 13$  probe. To determine the extent of the 5' sequence present in these  $G\alpha 12$  clones, PCR was done on the primary positive clones with oligonucleotide primers specific to the cloning vector and to the known  $G\alpha 12$  sequence (16). Those clones yielding the largest amplified fragments were purified. The complete sequences of two cDNAs encoding  $G\alpha 12$  and  $G\alpha 13$  are shown in Fig. 1. The predicted translation products are 379 and 377 amino acids in length, respectively. Both proteins are predicted to have a molecular mass of 44 kDa. A small open reading frame (12 amino acids) is located just upstream of the  $G\alpha 12$  coding region; however, the first methionine lies in a poor context for translation initiation (18).

The amino termini of  $G\alpha 12$  and  $G\alpha 13$  are quite distinctive when compared with other mammalian  $\alpha$  subunits (Fig. 2). A number of proteins, including  $G\alpha_i$  and  $G\alpha_o$ , are known to be N-myristoylated on a glycine at the second position (Gly-2, ref. 19). This modification is essential for anchoring the *src* oncogene product to the membrane (20). A comparison of the

known N-myristoylated proteins yields the consensus sequence Met-Gly-Xaa-Xaa-Xaa-Ser (2). Thus  $G\alpha 12$  and  $G\alpha 13$  are not predicted to be modified in this way. Perhaps these two  $\alpha$  subunits undergo a different form of posttranslational modification.

The crystal structures of ras and elongation factor Tu, as well as genetic studies of the effects of specific mutations on GTPase function, have revealed several domains in these proteins critical for guanine nucleotide interactions (5). Analogous regions in G protein  $\alpha$  subunits are readily identifiable (2). These regions are indicated in Fig. 2 as A, C, G, and I from Halliday's nomenclature (21). The function of the I region is not well characterized in the  $\alpha$  subunits; however, it is very highly conserved. All previously known mammalian  $\alpha$  subunits contain the sequence Thr-Cys-Ala-Thr-Asp-Thr, except  $G\alpha_s$  and  $G\alpha_{olf}$ , which differ at a single position (Fig. 2). A mutation (Thr-144 to Ile) in the analogous region in ras reduces GTP affinity by 25-fold (22). The fact that both  $G\alpha 12$  and  $G\alpha 13$  differ markedly from the consensus sequence in this region suggests that they may interact with guanine nucleotides in a manner distinct from some of the more well characterized  $\alpha$  subunits.

The carboxyl terminus of several  $\alpha$  subunits can be ADP-ribosylated by pertussis toxin. The modification occurs at a cysteine four residues from the end of the protein. Those mammalian  $\alpha$  subunits that lack this cysteine,  $G\alpha_s$ ,  $G\alpha_{olf}$ ,  $G\alpha_z$ ,  $G\alpha_q$ , and  $G\alpha 11$ , are all refractory to modification by pertussis toxin (2, 12, 23). The predicted amino acid sequences of  $G\alpha 12$  and  $G\alpha 13$  also lack this cysteine. Consequently, the translation products are likely to be insensitive to pertussis toxin.

**Distribution of  $G\alpha 12$  and  $G\alpha 13$  Messages.** RNA analysis indicates that the  $G\alpha 12$  message is  $\approx 4$  kilobases (kb), whereas the transcript encoding  $G\alpha 13$  is  $> 6$  kb (14). As a sensitive assay for the distribution of these transcripts in a wide variety of tissues, we used PCR RNA analysis (T. M. Wilkie and M.I.S., unpublished work). With this technique, multiple  $\alpha$  subunit sequences are amplified by PCR from cDNA by using degenerate oligonucleotide primers. These primers correspond to amino acid domains that are conserved in all  $\alpha$  subunits. An individual sequence is detected by Southern hybridization of the amplified pool with a labeled oligonucleotide specific for that sequence. The method provides a sensitive means of detecting the presence and relative abundance of a specific  $\alpha$  subunit sequence (Fig. 3). Both  $G\alpha 12$  and  $G\alpha 13$  can be detected in every tissue examined.  $G\alpha 12$  is expressed at relatively constant levels in most

$G\alpha 12$	MSGVVRTLSRCLLPAAEAGARRRAGAARDAREARRSRDIDALLARERRARRVRLVKILLGAGESGKSTFLKQMRIIHGREFDQK-ALLEFRDTIFDNILKGSRLVLDVA	A
$G\alpha 13$	MADFLPSRSVLSVCFPGCVLTNG-A-QQ-K-KE-KC-S-KTY-K-QD-R-RE-P-P-YS-VI-M	
$G\alpha_s$	MGCLGNSKTEDQNE-KAQ-EANKK-EKQ-QKDRQVY-ATHR-IV-L-VNG-NGESEKATKQVD-KN-LKEAETI-A	
$G\alpha 11$	MGCTLSAEDKA-VE-KM-RN-REDGEKAA-E-L-IV-K-EAGYSEE-ECKQYKAVVYS-TTQSIIAIR	
$G\alpha_q$	MTLESIMACCLSEEA-K-IND-E-ERHVR-DK-DA-EL-L-T-I-SGYSDE-DKRG-TKLQVYQ-F-TAMQAMIR	
$G\alpha 12$	RDKLGIPI-WQHSENEKHGMFLMAFENKAGLP-VEPATFQL-VPALSALWRDGSIREAFSRSEFQLGESVKYFLDNLDRIQQLNYFPSKQDILLARKATKGIVEHD	
$G\alpha 13$	E-H-GDNK-QL-DK-DTR-PMAGQM-TRV-LQ-L-IR-E-QN-YD-R-QD-R-RE-P-P-YS-VI-M	
$G\alpha_s$	MSN-VP-VELANP-QFRVDYILSV-MVPNDFPP-EF-EH-K-E-E-V-ACYE-SN-Y-IDCAQ-K-V-K-AD-V-D-L-RC-VL-S-F-TK	
$G\alpha 11$	MGR-K-DFGDAARADDARQL-VL-GAAEE-F-M-AE-AGVIKR-K-VQAC-N-SR-Y-ND-AA-Y-ND-A-P-I-TQ-V-RT-VK-T-TH	
$G\alpha_q$	M-T-K-YKYE-NKAHAQLVREVDV-KVSAFE-NP-D-IKS-N-P-Q-CYD-R-Y-SD-T-Y-ND-VADES-L-TQ-V-RV-VP-T-I-Y	
$G\alpha 12$	FVIKIPFKMVDVGGQSRQKWFQCFDGITSILFMVSSSEYDQVLMEDRRTRNLVSMNIFETIVNKLIFNVSIILFNKMDLLVEKVK-SVSIKKHFPDF	C
$G\alpha 13$	E-NV-E-KR-E-SV-L-F-Q-T-L-RV-S-KL-VPD-I-Q-RP-H-Y	
$G\alpha_s$	QVD-VN-H-F-DE-R-INDV-A-I-V-A-S-NM-IR-NQ-Q-AL-L-KS-W-RWLRTI-V-Q-A-LAGKSK-EDY-E-ARYTTP	
$G\alpha 11$	TF-DLH-F-E-K-IH-E-V-A-I-C-AL-D-L-A-EEM-MH-KL-DS-C-W-TDT-E-K-F-I-KSPLTICY-EY	
$G\alpha_q$	DLQSVI-R-E-R-IH-ENV-M-L-AL-V-SDNE-ME-KAL-R-ITYPW-Q-S-V-K-E-IM-YSHLVDY-EY	
$G\alpha 12$	-----KGDPHRLVDQRYLVQCFDR-KRRN-RSKPLFHHFTTAIDTENIRFVFAVKDTILQENLKDIMLQ	I
$G\alpha 13$	-----E-C-R-KF-E-RG-DQQR-Y-N-L-RD-HD-QL	
$G\alpha_s$	EDATPEP-EDP-VTRAKYFIRDE-L-ISTASGDGRHYCYP-C-V-R-NDCR-I-QRMH-RQYE-L	
$G\alpha 11$	-----A-S-NTY-EAAA-IQCQ-EDLN-KDTKEI-YT-C-T-K-VQ-D-T-V-IKN-CG-F	
$G\alpha_q$	-----D-PQRDAQAAREFILKM-VDLNFDSD-KII-YS-C-T-A-L-EYN-V	

FIG. 2. Amino acid comparison of  $G\alpha 12$  and  $G\alpha 13$ ,  $G\alpha_s$ ,  $G\alpha 11$ , and  $G\alpha_q$ . Dots represent identities with the  $G\alpha 12$  sequence. Boldface letters above the sequences identify conserved sequences.

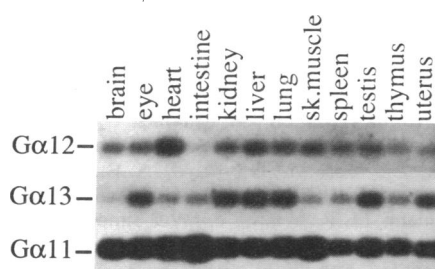


FIG. 3. PCR RNA analysis of  $G\alpha_{12}$ ,  $G\alpha_{13}$ , and  $G\alpha_{11}$ . RNA from various mouse tissues was reverse transcribed and PCR-amplified by using the degenerate oligonucleotides oMP19, oMP20, oMP21, and CT56. Amplified products were hybridized with radiolabeled oligonucleotides specific to  $G\alpha_{12}$ ,  $G\alpha_{13}$ , and  $G\alpha_{11}$ .

tissues, although it is noticeably lower in intestine.  $G\alpha_{13}$  appears to be expressed at somewhat higher levels in eye, kidney, liver, lung, and testis.  $G\alpha_{11}$  has been shown by RNA hybridization to be expressed at fairly constant levels in a variety of tissues (10); this pattern is reproduced by the PCR RNA analysis. Controls to ascertain the absence of contaminating chromosomal DNA were negative (data not shown, see *Materials and Methods*). It should be noted that PCR RNA analysis can minimize the effect of partially degraded RNA preparations, which upon RNA analysis will indicate artificially low expression levels. This effect is particularly pronounced with large messages and may explain the lower relative expression level originally observed for  $G\alpha_{13}$  in liver (14).

**Relationships Among the  $\alpha$  Subunits.** The previously known mammalian  $\alpha$  subunits can be grouped by amino acid identity into three classes:  $G_s$ ,  $G_i$ , and  $G_q$  (10). Members of one class, in general, are <50% identical to members of the other classes. These relationships represent not only primary sequence homologies but also, to some extent, functional similarities. Thus, within a class members often display significant levels of "crosstalk" in reconstitution experiments. For example, both  $G\alpha_s$  and  $G\alpha_{olf}$  can couple  $\beta$ -adrenergic receptors to adenylate cyclase (24). Also, the three types of  $G\alpha_i$  proteins, both purified and recombinant forms, can open atrial potassium channels (25).

$G\alpha_{12}$  and  $G\alpha_{13}$  define a fourth class of  $\alpha$  subunits (Fig. 4). They are <45% identical to members of the other three

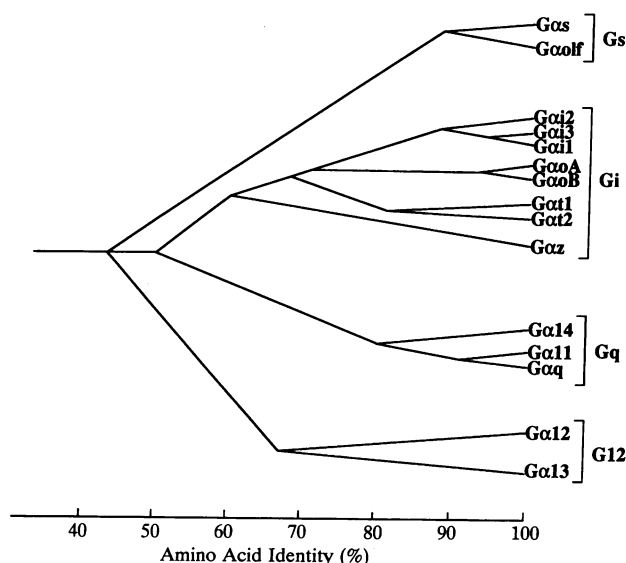


FIG. 4. Relationships among mammalian G protein  $\alpha$  subunits.  $\alpha$  subunits are grouped by amino acid identity (2, 10, 26, 27). Branch junctions approximate values calculated for each pair of sequences.

classes, but they share 67% amino acid identity with each other.

## DISCUSSION

Cellular responses elicited by numerous different ligands can be affected by pertussis toxin. The ability to inhibit a particular pathway with this toxin has been widely used to implicate a G protein mediator. Only the  $G_i$  class includes  $\alpha$  subunits known to be substrates for ADP-ribosylation by pertussis toxin. In fact, most genes encoding  $\alpha$  subunits yield products that appear insensitive to this modification. The ubiquitously expressed  $G\alpha_{12}$  and  $G\alpha_{13}$  cDNAs reveal a fourth class of  $\alpha$  subunits that are predicted to encode pertussis toxin-resistant proteins.

PLC-catalyzed release of inositol triphosphate and diacylglycerol was inferred to involve a pertussis toxin-resistant G protein that has been called " $G_p$ " (9). Purified activated  $\alpha$  subunits corresponding to the  $G\alpha_q$  and/or  $G\alpha_{11}$  clones have been shown to stimulate PLC- $\beta$  activity and to have some of the properties previously ascribed to  $G_p$  (13). However, there are a variety of PLC isoforms; many cells contain multiple related but distinct PLC gene products (28). Perhaps  $G\alpha_{12}$  and  $G\alpha_{13}$  couple to these other PLC isozymes or to other phospholipases that are activated through G-protein-mediated pathways.

The relationships among the  $\alpha$  subunits depicted in Fig. 3 developed early in the evolution of the animal kingdom. Representatives of the  $G_s$ ,  $G_i$ , and  $G_q$  classes have been found in *Drosophila* and nematodes (refs. 10, 29–36, U.-J. Kim, and M.I.S., unpublished work). It seems likely that the  $G_{12}$  class will be found in these organisms as well. In fact, Parks and Wieschaus (37) recently showed that the *Drosophila* gene *concertina* (*cta*) encodes a G protein  $\alpha$  subunit. *cta* is clearly more related to  $G\alpha_{12}$  and  $G\alpha_{13}$  (56% amino acid sequence identity) than to the other  $\alpha$  subunits (35–44% identity). Mutations in *cta* affect gastrulation and have a maternal effect; mothers homozygous for these mutations will survive, but their offspring do not develop properly. If the functions of specific  $\alpha$  subunits are conserved in evolution, then we may expect a member of the  $G_{12}$  class, perhaps as yet unidentified, to influence early developmental processes in more complex organisms.

The application of molecular biological techniques to the study of G proteins has proven to be a powerful tool in the analysis of signal transduction in complex multicellular organisms. The notion that the diverse signaling requirements of mammals need only a small set of G proteins is being replaced by the perception of greater complexity. In addition to multiple classes of  $\alpha$  subunits, extensive diversity of  $\beta$  and  $\gamma$  subunits has also been found (38–41). If combinatorial associations of the three subunits can occur in a functionally relevant manner, then the potential for G protein diversity is enormous.

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